

Local Lactate Perfusion of the Ventromedial Hypothalamus Suppresses Hypoglycemic Counterregulation

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We have previously reported that a glucosensor integrating hormonal responses to hypoglycemia is located in the ventromedial hypothalamus (VMH) and that local VMH glucose perfusion blocks counterregulatory hormone responses. To determine whether the by-product of glucose metabolism, lactate, can function within the VMH as an alternative for glucose, we delivered lactate locally to the VMH, during systemic hypoglycemia. For this purpose, we combined bilateral VMH microdialysis perfusion (metabolically active L-lactate or its nonmetabolizable D-isomer) with a euglycemic-hypoglycemic clamp in conscious chronically catheterized Sprague-Dawley rats. Local VMH perfusion with L-lactate decreased counterregulatory hormone responses to hypoglycemia by 80–85% as compared with the nonmetabolizable D-lactate control. Moreover, hormonal suppression with L-lactate was accompanied by an approximate fourfold increase in the amount of exogenous glucose infused to maintain a stable hypoglycemic plateau ($P < 0.05$). In conclusion, the glucose-sensing mechanism in the VMH responds to lactate and, thus, is not specific for glucose. This implies that the VMH may act as a fuel sensor rather than as a glucose sensor. *Diabetes* 52:663–666, 2003

Data demonstrating the importance of the central nervous system in hypoglycemia detection and the activation of glucose counterregulation (1,2) have generated interest in the location of and the mechanisms used by glucose-sensing neurons to trigger hormonal responses. While it is likely that several brain regions as well as extracerebral glucose-sensing neurons are involved (3–7), there is long-standing evidence supporting a central role for the hypothalamus, particularly the ventromedial hypothalamus (VMH), which is also recognized as a regulator of food intake (8–11). Previous studies have shown that electrical stimulation of the VMH (12) or local delivery of the nonmetabolizable glucose analog, 2-deoxyglucose, into the VMH (13) provokes the release of counterregulatory hormones. Con-

versely, focal lesioning of the VMH (14) or delivery of glucose by microdialysis directly into the VMH (15) suppresses counterregulatory responses to systemic hypoglycemia.

The mechanisms used by neurons to detect hypoglycemia remain to be established. It has been proposed that glucose-sensing brain regions contain neurons that are capable of altering their activity in response to changes in glucose levels. More than three decades ago, Oomura et al. (4) identified glucose responsive neurons in the VMH. Subsequently, neurons that are activated or inhibited by glucose have been described in the hypothalamus, brain stem, and the portal vein (6–9). In vitro studies have reported that the byproduct of glucose metabolism, lactate, can replace glucose as a fuel recognized by glucose-sensing neurons (8). In keeping with this possibility, it has been demonstrated that isolated neurons preferentially use lactate over glucose (16–18) and that infusion of lactate reduces the counterregulatory response to hypoglycemia (19,20).

The current study was undertaken to determine whether the VMH region responds only to glucose or whether other fuels can substitute for glucose in vivo. Specifically, we examined whether lactate infusion directly into the VMH blocks the counterregulatory hormone responses to systemic hypoglycemia in the awake rat.

RESEARCH DESIGN AND METHODS

Animals. Male Sprague-Dawley rats were purchased from Charles River Laboratories (Raleigh, NC). Animals were housed in an environmentally controlled room with a 12-h light/dark cycle and were maintained on standard ad libitum rat diet (Agway Prolab 3000; Agway, Waverly, NY) comprised of 22% protein, 5% fat, and 51% carbohydrate (the remaining 22% consists of ash, crude, fiber, and moisture).

VMH microdialysis cannula placement. Rats (250–315 g) were anesthetized by intraperitoneal injection (1 ml/kg) of a mixture of Xylazine (AnaSed 20 mg/ml; Lloyd Laboratories, Shenandoah, IA) and Ketamine (Ketaset 100 mg/ml; Aveco Co., Fort Dodge, IA) in a ratio of 1:2 (vol:vol) and placed on a stereotaxic frame. Thereafter, the skull was exposed, and holes were drilled bilaterally in chosen coordinates, through which the guide cannulas were lowered slowly into the brain. The stereotaxic coordinates were 2.6 mm posterior and 3.8 mm lateral in relation to bregma, and the angle was 20° in relation to the horizontal plane passing through bregma and lambda (21). The cannulas were then secured to the skull with stainless steel screws and dental acrylic. Animals were allowed to recover from the stereotaxic procedure for 12–16 days before study. One day before each experiment, microdialysis probes of side-by-side design (22) were inserted into the guide cannulas. The length of the probes was 10.5 mm, as measured from the bregma-lambda plane. The exposed microdialysis membrane was 1.0–1.5 mm (approximately the size of the VMH). On the morning of the experiment, the perfusion medium was loaded into 1-ml syringes and delivered at a flow rate of 2.5 μ l/min using a Harvard perfusion pump (model 22; Harvard Bioscience). In two groups of rats, the VMH was perfused bilaterally with a sterile, ascorbate-free, artificial, extracellular fluid solution (ECF) (135 mmol/l NaCl, 3 mmol/l KCl, 1 mmol/l

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Received for publication 12 September 2002 and accepted in revised form 17 December 2002.

ECF, extracellular fluid solution; VMH, ventromedial hypothalamus.

TABLE 1
Basal plasma hormonal and weight data of the animals studied

	VMH perfusions with:	
	L-lactate	D-lactate
<i>n</i>	5	6
Weight (g)	290 ± 20	280 ± 10
Insulin (nmol/l)	0.12 ± 0.03	0.12 ± 0.01
Glucagon (pg/ml)	176 ± 33	180 ± 20
Epinephrine (pg/ml)	226 ± 81	177 ± 38
Norepinephrine (pg/ml)	432 ± 60	317 ± 47

Data are means ± SD.

MgCl₂, 1.2 mmol/CaCl₂, and 2 mmol/l sodium phosphate buffer to pH 7.4). In one group, 100 mmol/l L-lactate (metabolically active isomer) was added to the perfusate, and in the other, 100 mmol/l D-lactate (nonmetabolizable isomer) was added to the perfusate to control for any potential osmotic effects of the perfusate used in the experimental group. A relatively high concentration of lactate was used in the perfusate because the efficiency of lactate delivery from these small probes is very low. We have previously estimated that the delivery of glucose via these probes *in vivo* is 1–2% (14), and the relative efficiency of delivery of lactate and glucose across the probe membrane is similar; specifically, it is only slightly greater for lactate (10–20%) than it is for glucose (unpublished data).

At the end of each experiment, the accuracy of probe placement was confirmed histologically by cresyl violet staining. Only those animals that showed bilateral probe placement into the desired brain region were analyzed (~25% of rats failed to meet histological criteria). The ability of the microdialysis system to deliver solutions to the VMH has been validated previously in our laboratory, using radiolabelled 2-deoxyglucose (13).

Surgical procedures. At 6–8 days before study (i.e., ~7 days after stereotaxic surgery), animals underwent an additional aseptic surgical procedure for placement of internal jugular vein and carotid artery catheters under intraperitoneal pentobarbital anesthesia (Nembutal, 35 mg/kg body wt; Abbott Laboratories, North Chicago, IL). The carotid artery catheter was extended to the level of the aortic arch, and the silicone internal jugular vein catheter was advanced to the level of the right atrium. At the end of the procedure, both catheters were flushed and filled with heparin 942 units/ml and polyvinylpyrrolidone (1.7 g/ml) solution, plugged, tunneled subcutaneously around the side of the neck, and externalized behind the head through a skin incision. Only those animals that had completely recovered were used in the studies.

Euglycemic/hypoglycemic clamp. The hyperinsulinemic-hypoglycemic clamp technique, as adapted for the rat (23), was used to provide a standardized hypoglycemic stimulus. Each of the animals was fasted for ~24 h before the study. On the morning of the experiment, the catheters were flushed and maintained patent by a slow infusion of saline (20 µl/min) that contained a small amount of heparin (1–2 units/ml). Animals were fully awake and freely moving about in their cages, unethered. After a 60-min rest period, basal blood samples were withdrawn. Thereafter, a primed (2,160 pmol over 1.5 min) continuous infusion (120 pmol · kg⁻¹ · min⁻¹) of porcine insulin (Eli Lilly & Co., Indianapolis, IN) was initiated and maintained for 150 min. A variable infusion of exogenous glucose was adjusted based on plasma glucose measurements obtained at 5-min intervals. During the first 60 min, the rats were maintained at euglycemia (mean plasma glucose 6.2 mmol/l). Thereafter, plasma glucose was allowed to fall over ~30-min period to hypoglycemic levels (~2.5 mmol/l) and then clamped at this level for ~60 min. Experiments were terminated if the plasma glucose 1) fell below 4.5 mmol/l during the last 45 min of the euglycemic phase and 2) rose above 3.9 mmol/l (secondary to glucose infusion) or inadvertently fell below 2.0 mmol/l during the hypoglycemic phase. Only the studies during which the mean blood glucose level achieved during hypoglycemia was in the range of 2.3–2.6 mmol/l were prospectively analyzed.

Blood samples for measurement of plasma insulin, glucagon, and catecholamines were taken during the euglycemic (30- and 60-min) and hypoglycemic (120-, 135-, and 150-min) phases of the experiments. Blood recently obtained from littermates was transfused during the clamp to quantitatively replace the blood withdrawn during the experiment. The protocol was reviewed and approved by the Yale Animal Care and Use Committee.

Analytical methods and calculations. Plasma glucose concentration was measured in duplicate using a Beckman Glucose Analyzer II (Beckman, Fullerton, CA). Plasma insulin (Binax, South Portland, MA) and glucagon (CN Biomedicals, Inc., Carson, CA) concentrations were determined by a double-antibody radioimmunoassay. Insulin measurements during the basal and insulin infusion periods were made using rat and porcine standards, respec-

TABLE 2
Mean plasma glucose concentrations during euglycemia and hypoglycemia

	VMH perfusions with:	
	L-lactate (n = 5)	D-lactate (n = 6)
Euglycemic phase		
Glucose (mmol/l)	6.2 ± 0.1	6.3 ± 0.2
Hypoglycemic phase		
Glucose (mmol/l)	2.4 ± 0.2	2.4 ± 0.1
Exogenous		
Glucose infusion rate (mg · kg ⁻¹ · min ⁻¹)	5.4 ± 1.2*	1.4 ± 0.3

Data are means ± SD. Glucose values were obtained by averaging measurements during the 0–60 min and 90–150 min of the study. **P* < 0.05.

tively. Plasma catecholamines were measured with a radioenzymatic method (Amersham, Arlington Heights, IL). Data are expressed as mean ± SE. Comparison between the experimental groups was made by ANOVA with a repeated measure design, followed by the Student's *t* test to localize effects. Glucose infusion rates during the hypoglycemic steps represent average values for the designated time interval.

RESULTS

At the outset of the study, neither the basal plasma concentrations of glucoregulatory hormones nor the weight of the animals (Table 1) were significantly different in both animal groups. During the insulin infusion, plasma insulin rose to nearly identical levels (4.7 ± 0.5 nmol/l L-lactate vs. 4.6 ± 0.3 nmol/l D-lactate), and plasma glucose was comparable during each phase of the study in all groups (Table 2). In the hypoglycemic phase of the study, the desired level of glycemia (i.e., ~2.5 mmol/l) was achieved after ~30 min.

During euglycemia, the concentrations of epinephrine, norepinephrine, and glucagon were not significantly different between the two experimental groups of animals. The effects of the VMH microdialysis perfusions on counter-regulatory hormones to hypoglycemia are summarized in Fig. 1. In the experimental group (VMH perfused with 100 mmol/l L-lactate) hormonal responses were diminished, despite a similar degree of systemic hypoglycemia. Specifically, 100 mmol/l L-lactate perfusion into the VMH suppressed the release of epinephrine and norepinephrine by

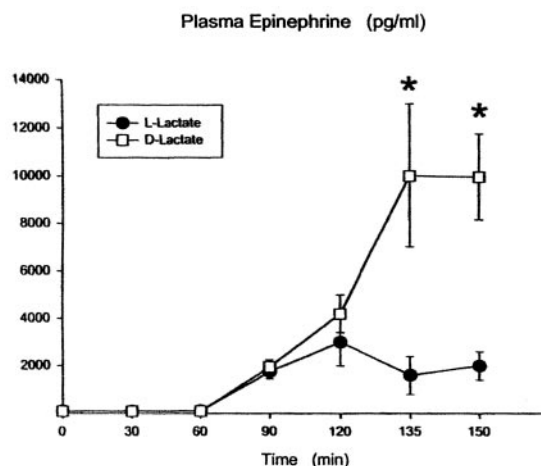


FIG. 1. Effect of L-lactate and D-lactate VMH perfusion on the plasma epinephrine response to systemic hypoglycemia.

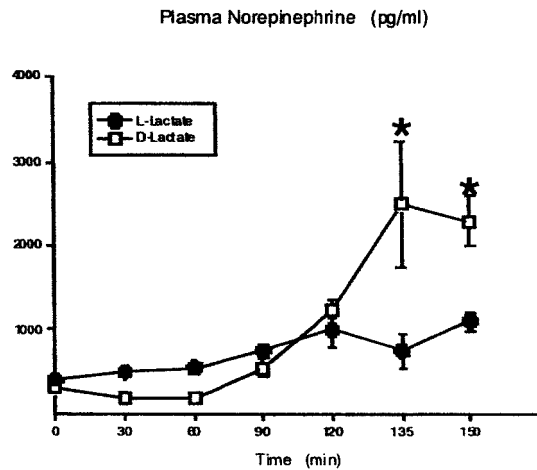


FIG. 2. Effect of L-lactate and D-lactate VMH perfusion on the plasma norepinephrine response to systemic hypoglycemia.

90% and glucagon by 85% ($P < 0.05$ as compared with the control group). In contrast, there was a sharp increase in the concentrations of plasma catecholamines and glucagon in the control group, when hypoglycemia was induced.

The diminished counterregulatory hormone responses in the animals receiving VMH L-lactate perfusion was associated with an approximate fourfold higher rate of exogenous glucose infusion than was required in the control studies to maintain the hypoglycemic plateau (Table 2).

DISCUSSION

This study demonstrates that when lactate, a by-product of anaerobic glucose metabolism, is delivered selectively into the VMH, the catecholamine and glucagon responses are reduced in the face of peripheral hypoglycemia. The delivery of lactate to the VMH also increased the requirement for exogenous glucose to maintain the hypoglycemic plateau, in keeping with its suppressive effect on counterregulatory hormone responses.

The role of energy-yielding fuels other than glucose in brain metabolism during hypoglycemia is poorly understood. Yet, it has been reported that while the glucose metabolic rate in brain falls during hypoglycemia, there is

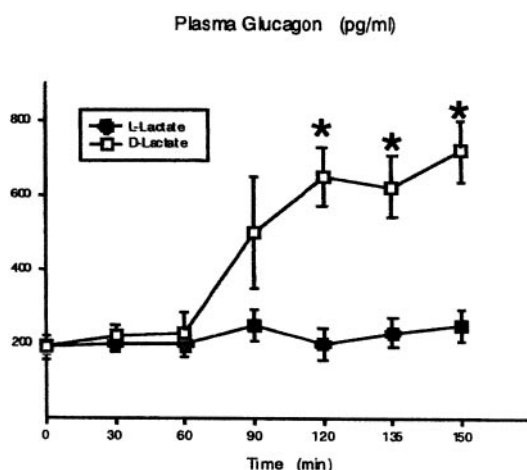


FIG. 3. Effect of L-lactate and D-lactate VMH perfusion on the plasma glucagon response to systemic hypoglycemia.

little change in the rate of oxygen consumption (24), implying the utilization of other fuels. Glycogen stores appear to be insufficient to sustain brain metabolism (24) and blood ketones decline during insulin-induced hypoglycemia, making them unlikely candidates. Recent studies suggest that lactate may serve as an important source of fuel. Specifically, both in vivo and in vitro studies suggest that lactate is readily utilized as a substrate for neurons when glucose is deficient (19,20,25,26). While blood levels of lactate and its transport across the blood-brain barrier appear not to be the major source of lactate supply to the brain (24,27,28), it has been reported that lactate is produced locally in brain by astrocytic nonoxidative glucose metabolism during neuronal activation (29,30). In keeping with this hypothesis, local stimulation of brain tissue in vivo produces a rise in brain interstitial lactate concentration in the absence of changes in systemic lactate levels (31). Moreover, while estimates of lactate concentrations using magnetic resonance spectroscopy in normal human brain (which includes intra- and extracellular fluid) are between <0.03 and 1 mmol/l (32), recent more direct measurements of lactate in brain extracellular fluid (ECF) in rodents and human subjects by microdialysis show a much different picture. Lactate levels in the brain ECF are three- to fivefold higher than plasma (28). In comparison, brain interstitial glucose concentrations, measured by microdialysis are estimated to be ~ 25 – 30% systemic glucose levels (28). Thus, there is considerable evidence suggesting that lactate is readily available to neurons within the central nervous system and that it can be used as an alternative fuel source, particularly when glucose becomes deficient.

In the current study, sufficient lactate was made available to neurons within the VMH region to inhibit normal activation of counterregulatory hormone responses, despite a 61% fall in plasma glucose, and in turn a similar fall in VMH interstitial glucose levels to values that are likely to be well below 1 mmol/l (28). A similar effect was not seen when the VMH was perfused with nonmetabolizable D-lactate. These data suggest that the glucose sensing neurons within the VMH are capable of utilizing lactate as well as glucose, and thus they appear to act as a fuel sensor rather than simply as a glucose sensor. These findings are consistent with evidence that the addition of lactate to brain slices stimulates the activity of glucose responsive neurons in VMH (8). Although it is likely that the increases in lactate achieved in the VMH are modest given the low efficiency of the probes used, the local concentrations produced in these experiments are uncertain and therefore it remains to be established that the observed effects of lactate would be seen in the physiological setting.

ACKNOWLEDGMENTS

This research was supported by grants from the National Institutes of Health, R37 DK 20495, R01 DK 40936, and P01 DK 45735. M.A.B. was a Fellowship grant recipient from the Juvenile Diabetes Research Foundation.

We are indebted to Dr. Michael Brines for his comments and suggestions. We appreciate the assistance of Aida Groszmann and Andrea Belous for their help in the measurements of plasma hormones.

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